Comparative analysis of the rheological properties of blood in patients with type 2 diabetes

V. Kostova\textsuperscript{a}, N. Antonova\textsuperscript{a}, I. Velcheva\textsuperscript{b}, I. Ivanov\textsuperscript{a}

\textsuperscript{a}Department of Biomechanics, Institute of Mechanics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; v.kostova@imbm.bas.bg; antonova@imbm.bas.bg

\textsuperscript{b}Department of Neurology, University Hospital of Neurology and Psychiatry “St. Naum”, Medical University. Sofia, Bulgaria; velchevairena@imbm.bas.bg

Abstract

The aim of the study is to estimate the rheological properties of blood in patients with type 2 diabetes and to compare the hemorheological parameters with a control group of healthy subjects. Hemorheological variables, including hematocrit (Ht), fibrinogen (Fib) and whole blood viscosity (WBV) at shear rates of 0.0237 s\textsuperscript{-1} to 128.5 s\textsuperscript{-1} were examined in 20 patients with diabetes mellitus type 2 and in 10 control subjects. Whole blood viscosity was measured using a rotational viscometer Contraves Low Shear 30 (Switzerland). Relationship between dynamic viscosity and Ht, Fib, Erythr and MCV were evaluated by coefficient of correlation “r”.

The results of this study confirm the significant increase of whole blood viscosity in the patients in comparison to controls within the range of shear rates 0.0237 s\textsuperscript{-1} to 128.5 s\textsuperscript{-1}, which is due to the increased values of hematocrit, fibrinogen and erythrocytes. Rheological abnormalities such as increased aggregation and decreased deformation of erythrocytes also contribute to increase of blood viscosity which leads to the vascular blood flow changes in diabetes.

Keywords: Type 2 diabetes, hemorheological properties, erythrocyte aggregation and deformation

1. Introduction

Diabetes mellitus is a disease characterized by elevated blood glucose in the body due to impaired response of cells to insulin in type 2 diabetes or its insufficient production in the body in type 1 diabetes. When cells do not absorb enough glucose, it accumulates in the blood (hyperglycemia), which leads to complications in the cardiovascular, nervous and other systems. Diabetes and its complications have a significant economic impact on individuals, families, health systems and countries. [1].

According to the International Diabetes Federation, 31 million people in Europe suffer from diabetes and the disease incidence is 8.6 %. The number of the people with diabetes in Bulgaria is about 595,700 [2]. People with type 2 diabetes have two to four times higher risk of coronary heart disease than the rest of the population, and their prognosis is poorer. The risk of cerebrovascular and peripheral vascular disease is also significantly higher. Premature mortality caused by diabetes results in an estimated 12–14 years of life lost [3].

1.1. Whole Blood Viscosity

The hemorheological parameters in diabetes mellitus are often disturbed. These parameters include (but are not limited to) hematocrit, plasma proteins, erythrocyte aggregation, and erythrocyte deformability. The abnormalities associated with each of these parameters have been shown to markedly increase both plasma and whole blood viscosity (WBV) [4].

The increase in blood viscosity may be particularly important in the etiology of diabetic retinopathy [5]. The etiology of diabetic microangiopathy may be a violation of the microcirculation, leading to continuous reduction in the supply of oxygen and nutrients to the capillary vessels [6]. Osmolarity of the blood also affects blood viscosity.
1.2. Red blood cell aggregation in diabetes

Red blood cells of patients with type 2 diabetes are associated in aggregates and aggregate more easily than those of healthy subjects. Red blood cell (RBC) aggregation has been widely studied and its importance is well established in the rheology of microcirculation [8]. RBC aggregation is a major factor responsible for the flow properties of blood. Increased RBC aggregation has been observed in several pathological states [9]. Erythrocyte aggregation is a reversible phenomenon responsible for increased blood viscosity at low shear rates [10]. In plasma, fibrous rather than globular proteins are responsible for aggregation, with fibrinogen concentration being the most important determinant of the aggregating property of plasma. Aggregation is considered the main cause of increased blood viscosity at low shear rates, while at higher shear rates the viscosity of blood is determined mainly by the deformation of erythrocytes [11].

2. Subjects and methods

2.1. Subjects

The study included 20 patients (16 women and 4 men, mean age 57.25 ± 5.81 years) with type 2 diabetes mellitus. The diagnosis of diabetes mellitus was based on clinical examination and estimation of blood glucose, insulin and HbA1C; its mean duration was 12.33 ± 5.3 years. Blood samples were collected in Li heparin tubes and rheological measurements were completed within 3 hours after blood preparation.

2.2. Methods

Whole blood viscosity was measured using a rotational viscometer Contraves Low Shear 30 (Switzerland) under conditions of steady blood flow at 11 shear rates from 0.0237 s\(^{-1}\) to 128.5 s\(^{-1}\) at 37°C. The results were compared with a control group of 10 healthy subjects (6 women and 4 men, mean age 54.90 ± 7.96 years) (Fig. 1) [12].

The examined hemorheological blood constituents were: hematocrit (Ht), erythrocytes, mean cell volume (MCV), fibrinogen (Fib) and whole blood viscosity. Taylor factor was calculated and used test "ANOVA" to determine the differences in mean values of the studied parameters of significance level of p <0.05. The relationship between dynamic viscosity and Ht, Fib, Erythr and MCV were evaluated using the correlation coefficient (r) (Fig. 5).

2.3. Equation

Taylor's formula, erythrocyte rigidity index (Eq. 1) [13].

\[ T_K = \frac{\eta_r^{0.4} - 1}{\eta_r^{0.4} \times Ht} \]

\[ \eta_r = \frac{\eta_{br}}{\eta_{pl}} \]

Ht is hematocrit, \( \eta_r \) is the relative viscosity, where \( \eta_{br} \) – apparent blood viscosity at a shear rate 51.2 s\(^{-1}\) and \( \eta_{pl} \) - plasma viscosity at a shear rate 94.5 s\(^{-1}\).

3. Results

In the patients with type 2 diabetes significant increase of Fib and erythrocytes (Table 1) and WBV at different
shear rates (Fig. 1.) in the patients’ group were found.

Table 1
Mean values and standard deviations of Ht, Erythr, MCV
and Fib in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Diabetes, mean ± SD (n= 20)</th>
<th>Controls, mean ± SD (n= 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht, %</td>
<td>42,58 ± 4,85</td>
<td>41,83 ± 2,60</td>
</tr>
<tr>
<td>Erythr 10^{9}/ml</td>
<td>5,64 ± 2,66*</td>
<td>4,75 ± 0,24</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>84,94 ± 8,79</td>
<td>88,08 ± 4,07</td>
</tr>
<tr>
<td>Fib, g/l</td>
<td>4,54 ± 0,83**</td>
<td>3,66 ± 0,67</td>
</tr>
</tbody>
</table>

* p<0.05; **p<0.01 - significance in comparison to controls.

Fig. 1 shows a comparison of the whole blood viscosity of the diabetics with the healthy control subjects within a wide range of shear rates. The observed increase in the mean values of whole blood viscosity in the studied group of patients with diabetes compared with healthy subjects at shear rates over the entire range were statistically significant at medium shear rates (0,512 s^{-1}; 1,285 s^{-1}).

Table 2
Mean values and standard deviations of dynamic viscosity values at different shear rates in patients and controls [12]

<table>
<thead>
<tr>
<th>Shear rate [s^{-1}]</th>
<th>( \eta_{\text{diabetes}} ) [mPa.s], mean± SD (n= 20)</th>
<th>( \eta_{\text{controls}} ) [mPa.s], mean± SD (n= 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,0237</td>
<td>83,22 ± 44,24</td>
<td>45,75 ± 2,15**</td>
</tr>
<tr>
<td>0,0596</td>
<td>55,60 ± 30,89</td>
<td>45,99 ± 13,52</td>
</tr>
<tr>
<td>0,1102</td>
<td>40,59 ± 13,29</td>
<td>38,12 ± 7,37</td>
</tr>
<tr>
<td>0,512</td>
<td>31,67 ± 8,30</td>
<td>14,71 ± 12,39**</td>
</tr>
<tr>
<td>1,285</td>
<td>20,62 ± 16,20</td>
<td>8,02 ± 5,82***</td>
</tr>
<tr>
<td>5,96</td>
<td>10,85 ± 2,72</td>
<td>8,57 ± 4,12</td>
</tr>
<tr>
<td>11,02</td>
<td>9,01 ± 1,76</td>
<td>8,23 ± 0,77</td>
</tr>
<tr>
<td>20,4</td>
<td>7,73 ± 1,40</td>
<td>7,11 ± 0,54</td>
</tr>
<tr>
<td>51,2</td>
<td>6,13 ± 0,89</td>
<td>5,83 ± 0,39</td>
</tr>
<tr>
<td>94,5</td>
<td>5,44 ± 0,69</td>
<td>4,97 ± 0,19</td>
</tr>
<tr>
<td>128</td>
<td>5,16 ± 0,57</td>
<td>4,65 ± 0,09</td>
</tr>
</tbody>
</table>

**p<0,05, ***p<0,01 - significance in comparison to controls.
At low shear rate, the whole blood viscosity is determined mainly by the RBC aggregation while at high shear rate it is determined by the deformability of RBCs. The RBC aggregation process is considered to be related to plasma fibrinogen and concentrations of globulins. Generally, the viscosity of blood depends on macrohemorheological parameters: hematocrit and serum proteins (fibrinogen and globulins) (Fig. 2) and microhemorheological parameters such as degree of erythrocyte aggregation and deformation.

**Fig.2.** Erythrocytes (Erythr) - a) and fibrinogen (Fib) - b) in control group (n = 10) and in patients (n = 20).

Significant differences for Fib (p <0.01) and Erythr (p <0.05) in patients with diabetes in comparison to healthy subjects is found. The elevation of Fib and Ht in diabetes is due to changes in the red cell count and behaviour. (Fig. 2a), b) and 3b)). As compared to controls the diabetic patients have lower mean cell volume (MCV) and higher Ht but the differences do not reach statistical significance.

Correlation analysis was used to explore quantitatively the relationship between the measured parameters (Fig. 4, 5).

**Fig.3.** Mean cell volume (MCV)-a) and hematocrit (Ht)-b) in control group (n = 10) and in patients (n = 20).

**Fig.4.** Linear distribution of the dynamic viscosity at different shear rates of hematocrit (Ht) for the diabetic group (n = 20).
Fig. 5. Coefficient of correlation between the dynamic viscosity at different shear rates (0.0237 s⁻¹; 0.512 s⁻¹; 5.96 s⁻¹; 94.5 s⁻¹) and hematocrit (Ht) for diabetic group.

The results show that there is a linear correlation between the dynamic viscosity (at a shear rate 0.0237 s⁻¹; 0.512 s⁻¹; 5.96 s⁻¹; 94.5 s⁻¹) and hematocrit (in the range of 33% to 53%) of the diabetic group.

The calculated erythrocyte rigidity $T_k$ index is higher in the patients with diabetes as compared to the healthy subjects.

4. Discussion

Variations of shear rates affect aggregation, deformation and orientation of RBCs, leading to changes in whole blood viscosity observed in patients and in control subjects. The whole blood viscosity is strongly influenced by the RBC aggregation at low shear rates while with increasing shear rates, the number and the size of aggregates is decreased. At low shear rate, the viscosity depends on the plasma fibrinogen too. In patients with diabetes an increase of fibrinogen, a predictive factor for the development of retinopathy and nephropathy is observed [4]. The elevated number of red blood cells in the patients group as compared with controls leads to increased aggregation of red blood cells. The data about increased dynamic viscosity in the group suffering from type 2 diabetes in comparison to the control subjects indicates reduced deformability of erythrocytes at high shear rates in the diabetics.

The results demonstrate a linear correlation between dynamic viscosity and hematocrit in diabetes. The linear correlation analysis indicates that the coefficient of correlation ($r$) ranged between $r = 0.1$ - 0.6 (up to 0.3 - weak to moderate; 0.5 to 0.7 - considerable) at shear rate = 0 0237 s⁻¹; 0.512 s⁻¹; 5.96 s⁻¹; 94.5 s⁻¹. This shows that the increase in viscosity follows the increase of the other hemorheological parameters (Ht, Erythr and Fib).

Acknowledgements

This work was done with the support of the Ministry of Education, Youth and Science of Bulgaria, Contract № Д 02-798/28.08.2012 within the framework of the project “Science and Business”.

References


